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Speculative article

Expression of endoglin in psoriatic involved and uninvolved skin

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Received 4 July 1994; revision received 25 November 1994; accepted 1 January 1995

Abstract

Endoglin is a glycoprotein with TGF- β binding capacity and is predominantly expressed on endothelial cells. In psoriasis, TGF- β has appeared to play a role in the extravasation of peripheral blood mononuclear cells via the endothelium. In order to find out more about the role of endoglin in psoriasis, immunohistochemical staining with PN-E2, a novel anti-endoglin, and of PAL-E, recognizing vascular endothelium, was carried out in psoriatic involved, psoriatic uninvolved and normal skin. The expression of the antigens was assessed semi-quantitatively using a five-point scale. In psoriatic involved skin, a high endoglin expression was found. In psoriatic uninvolved skin, however, we found that endoglin expression was significantly decreased compared with normal skin. The relevance of these findings to the pathogenesis of psoriasis is discussed.

Keywords: Psoriasis; PN-E2

1. Introduction

The role of the endothelium in the pathogenesis of psoriasis has been discussed by several authors

Abbreviations: DMEC, dermal microvascular endothelial cells; HEV, high endothelial venules; IL, interleukin; LTB₄, leukotriene B₄; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cells; PMN, polymorphonuclear leukocytes; TGF- β , transforming growth factor beta; TNF, tumor necrosis factor.

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[1–7]. In the chronic psoriatic plaque a tortuous dilated microvasculature constitutes the histological pattern which is familiar to every dermatopathologist. The ultrastructural changes of the endothelium consist of intrapapillary capillaries with a predominantly venous character and bridged fenestrations [1,2,4,5]. Other groups reported on so-called high endothelial venules (HEV) that form the site of preference for the binding of T-lymphocytes to endothelium and their subsequent migration into the surrounding tissue [8]. In the

marginal zone of spreading psoriatic plaques an increased bloodflow, assessed by laser Doppler flowmetry and an increase of endothelial alkaline phosphatase activity, has been reported to precede the appearance of the overt clinical lesion [9,10]. In the distant uninvolved skin Braverman and Sibley claimed to have observed vasodilatation [3], whereas Heng et al. reported the presence of HEVs which are absent in normal skin [8]. Also, the capillary resistance of the uninvolved skin in psoriasis appeared to be decreased compared with normal skin [11].

The aim of the present communication was to investigate to what extent endoglin expression coincides with psoriatic involvement and possibly find out more about its meaning. Expression of endoglin is considerably upregulated under inflammatory conditions and in skin lesions where endothelial cell proliferation occurs [12]. Endoglin is a glycoprotein which has been associated with adhesion processes [13] and which has transforming growth factor beta (TGF- β) binding capacity [14]. In psoriatic skin, the role of TGF- β is not yet completely clarified, although it was found that TGF- β does not influence the adhesiveness of the peripheral blood mononuclear cells to the dermal microvascular endothelial cells as it does in normal skin [15]. Monoclonal antibodies (mAbs) known to react with endoglin are RMAC8, HEC-19,

8E11, 1G2 and 44G4 [13]. However, they do not all recognize the same epitope. In this study PN-E2, a recently developed Ig-G1 monoclonal antibody [12], was used as a marker for endoglin. The mAb PAL-E was used as a marker for vascular endothelial cells [16,17].

2. Materials and methods

2.1. Volunteers

Six patients and 11 healthy persons volunteered and gave their informed consent for this study, which was carried out after approval of the local Medical Ethical Committee. The patient group consisted of 3 females and 3 males, aged between 28 and 63 years, whereas the group of healthy volunteers was composed of 4 females and 7 males, whose age varied from 17 to 63 years.

The patients had received neither local anti-psoriatic treatment for at least 2 weeks nor systemic treatment for at least 4 weeks prior to the study. None of the healthy volunteers had signs or a history of skin diseases.

2.2. Biopsies

From the involved and uninvolved psoriatic skin and from the normal skin 4 mm punch-biopsies were obtained after local infiltration with 1 ml Xylocain 1% with adrenalin 1:100.000 (Xylocain, Astra, Rijswijk, The Netherlands). After the

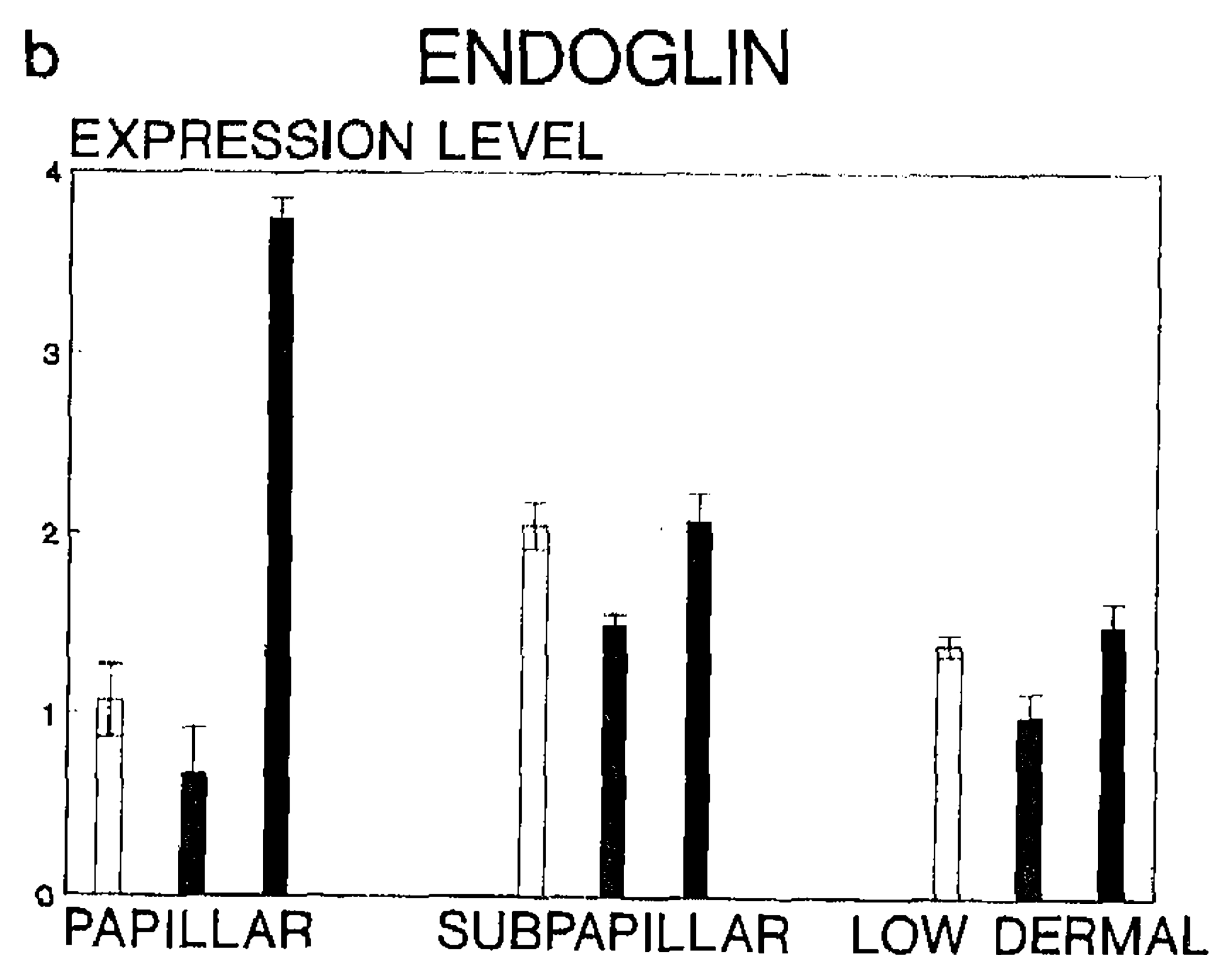
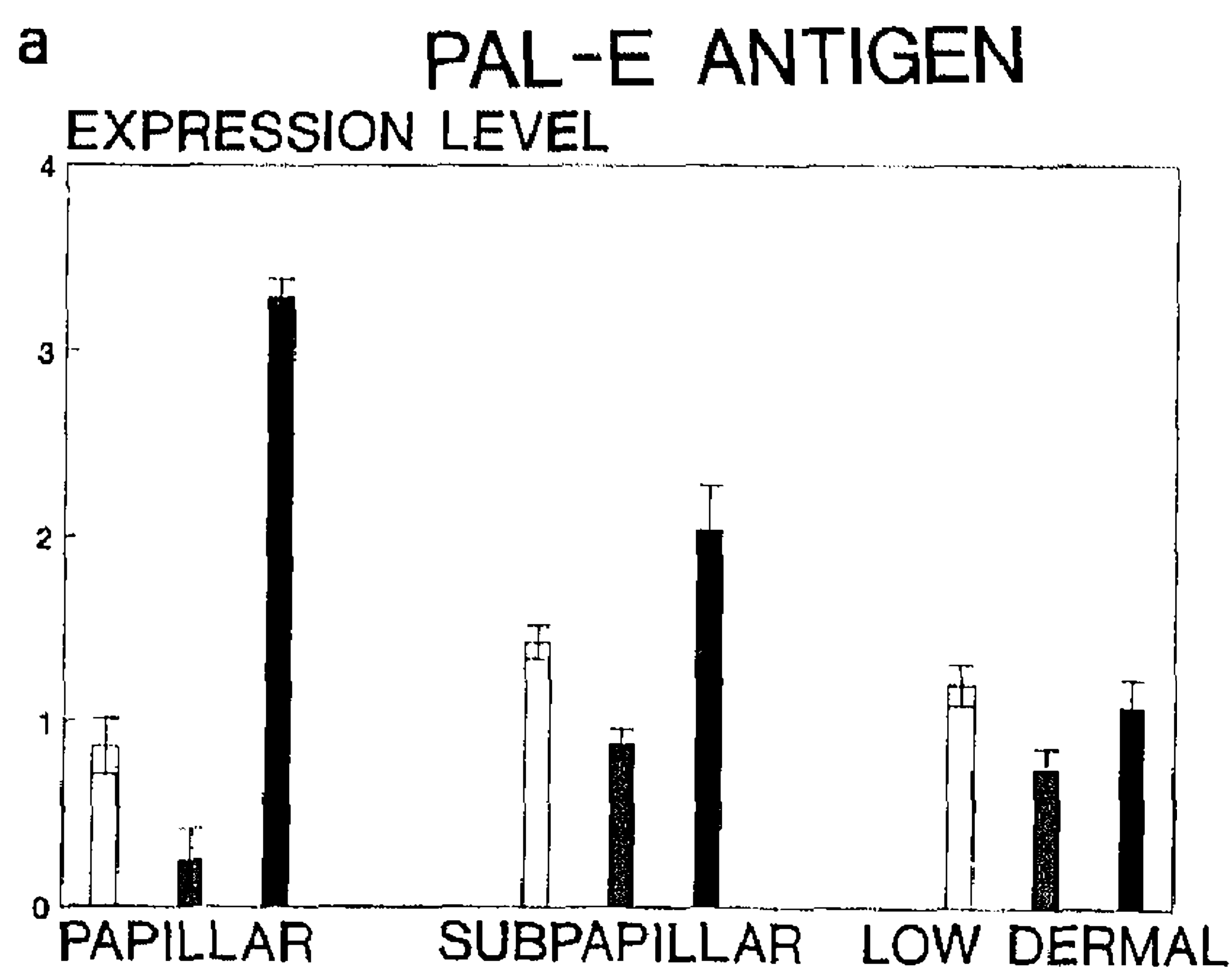


Fig. 1. Normal and psoriatic skin: a. level of PAL-E antigen expression, b. level of endoglin expression.

biopsies were imbedded in TissueTec OTC Compound (Miles Scientific, Naperville, IL), they were snap-frozen in liquid nitrogen and stored at -80°C . Subsequently, 7 μm frozen sections were cut and fixed in acetone for 10 min.

2.3. Monoclonal antibodies

The monoclonal antibody PAL-E (Department of Pathology, University Hospital Nijmegen) [16,17] was used (1:50 dilution) to visualize the endothelium of the microvasculature. This antibody specifically recognizes vascular endothelium and does not stain the endothelial lining of lymphatic vessels. To assess endoglin expression, the monoclonal antibody PN-E2 (Department of Pathology, University Hospital Nijmegen, The Netherlands) was used (1:20 dilution).

2.4. Staining procedures

Staining was performed using an indirect immunoperoxidase technique. Negative control stainings were included.

In brief, slides were rehydrated in phosphate-buffered saline (PBS) with Tween 80 and then incubated with a monoclonal antibody for 60 min. After three washes with PBS they were incubated for 30 min with peroxidase-conjugated rabbit-anti-mouse antibody (RAMPO, Dakopatts, Copenhagen, Denmark). Three more washes preceded a pre-incubation with sodium acetate buffer (pH 4.9), after which the slides were finally stained with freshly prepared 3-amino-9-ethyl-carbazole 200 mg/l in sodium acetate buffer and 0.01% H_2O_2 (AEC solution). All slides were finally washed with demineralized water, counterstained with Mayer's haematoxylin (Sigma, St. Louis, MO) and mounted in glycerine gelatine.

2.5. Histological examination

Slides were studied by light microscopy. Using a five-point scale, the staining pattern was assessed at the papillar, subpapillar and low dermal region. This subdivision corresponded with anatomical structures as described in [6,18].

2.6. Statistical analysis

The nonparametrical Mann-Whitney test was used for statistical evaluation.

3. Results

Preliminary semiquantitative assessments were carried out in order to establish the system of scoring. Fig. 2 illustrates the various degrees of expression.

In normal as well as in psoriatic uninvolved skin the distribution of the two markers over the three compartments showed a consistent pattern in which the most pronounced staining was seen in the subpapillar region (subpapillar plexus). Psoriatic involved skin, however, demonstrated a different pattern. In the papillar region of lesional skin, the degree of staining assessed by PAL-E and PN-E2 was markedly increased compared with normal and psoriatic uninvolved skin (Fig. 2). Furthermore, in some of the tips of the dermal papillae endoglin expression on the microvascular endothelium was more pronounced.

In the subpapillar and low dermal region of psoriatic uninvolved skin a statistically significant decrease in the occurrence of PAL-E staining was seen ($P = 0.016$ and $P = 0.027$ respectively, see Table 1). Remarkably, a statistically significant decrease in PN-E2 staining was seen at all levels of the psoriatic uninvolved skin compared with normal skin ($P = 0.031$, $P = 0.004$ and $P = 0.035$, see Table 1).

To determine whether this decrease was merely due to a lower vascularization grade in general or due to a relatively decreased amount of activated endothelium, the ratios of endoglin and PAL-E expression were estimated. This was done for all levels of the normal and the psoriatic uninvolved skin (see Table 2). As previously shown, an increased endoglin expression in dermal capillaries is associated with endothelial activation [12]. It was

Table 1

P-values (Mann-Whitney test) comparing immunohistochemical staining in normal skin and uninvolved skin of psoriatic patients

	Papillar	Subpapillar	Low dermal
PAL-E	0.315	0.016	0.027
PN-E2	0.031	0.004	0.035

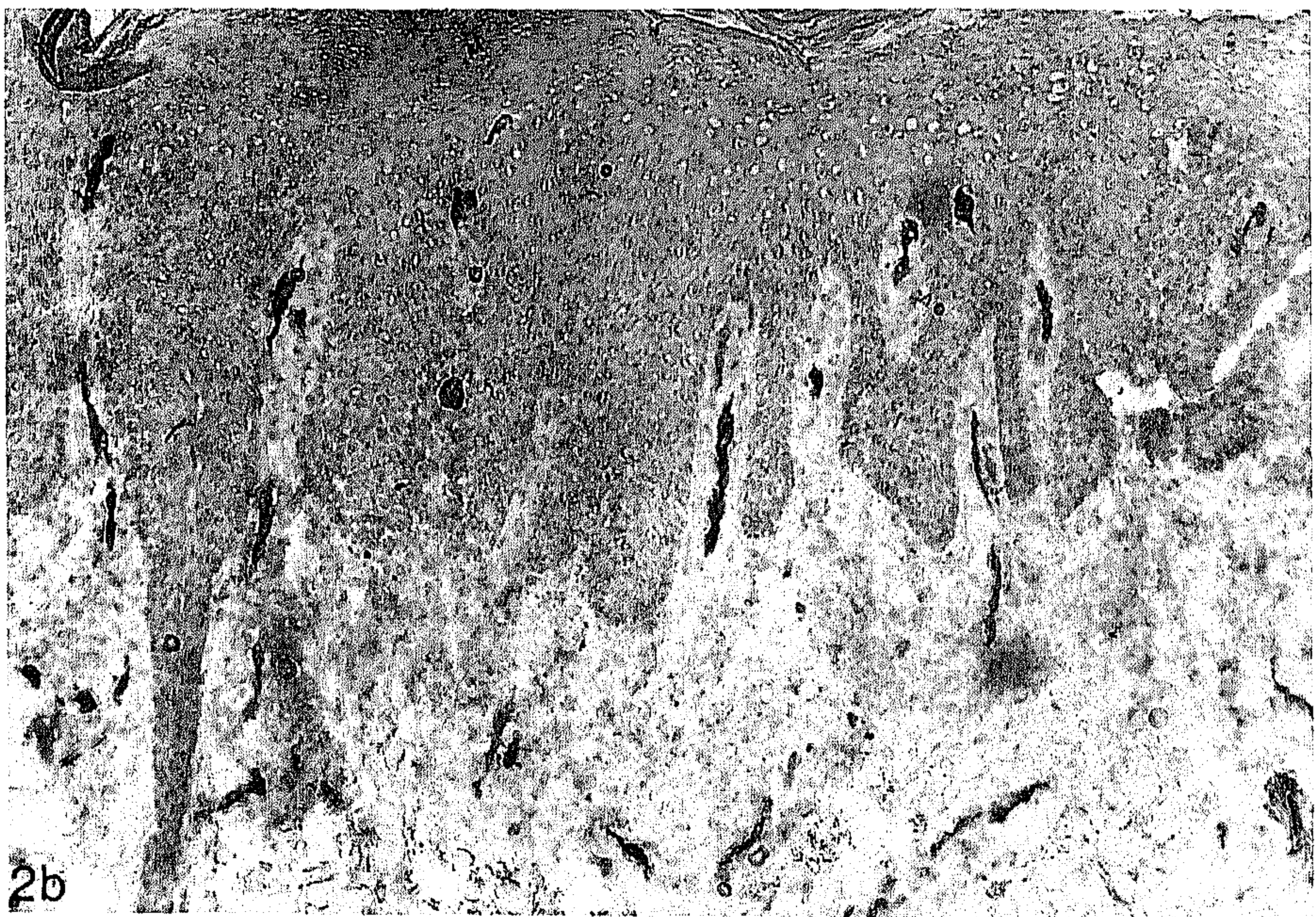
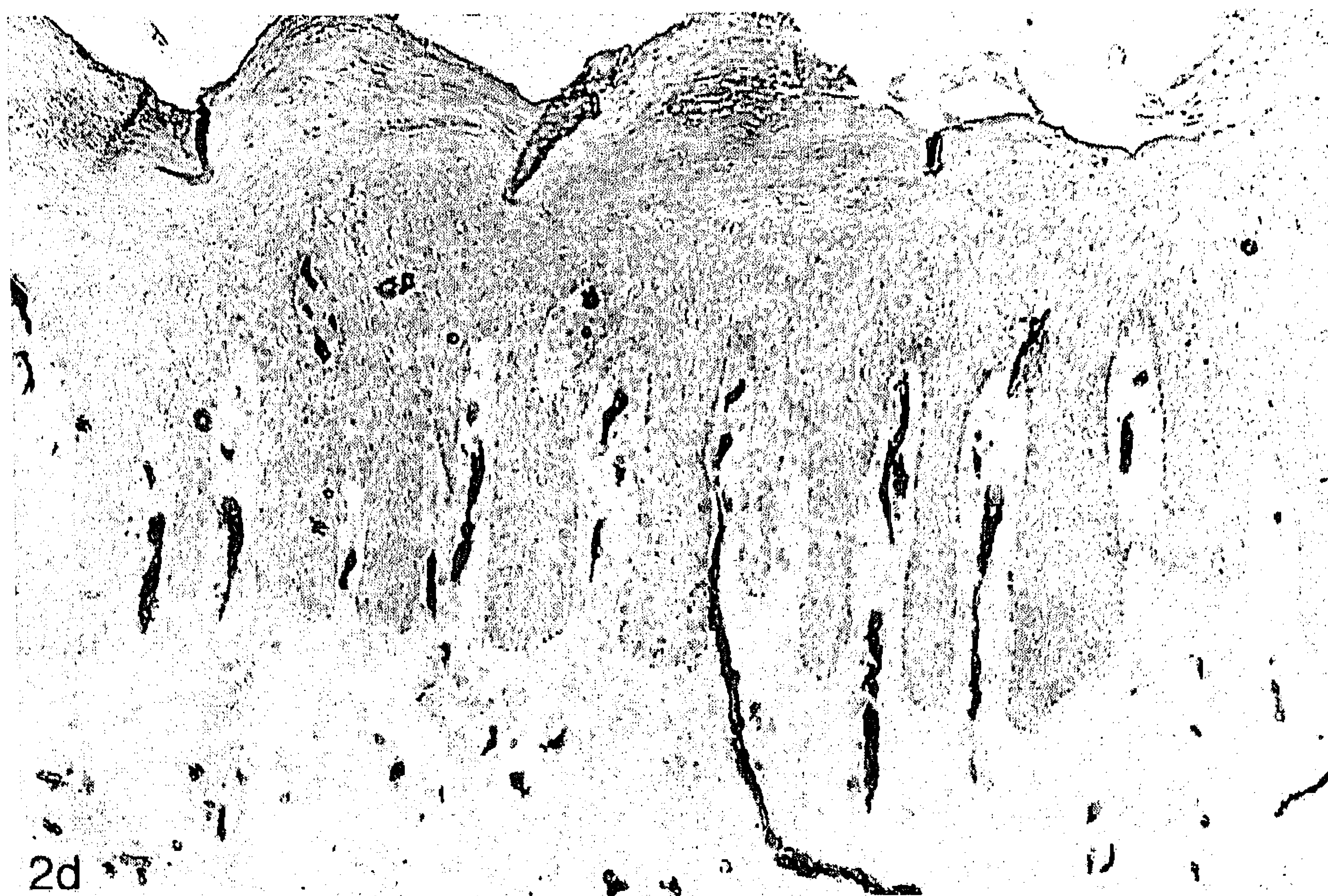
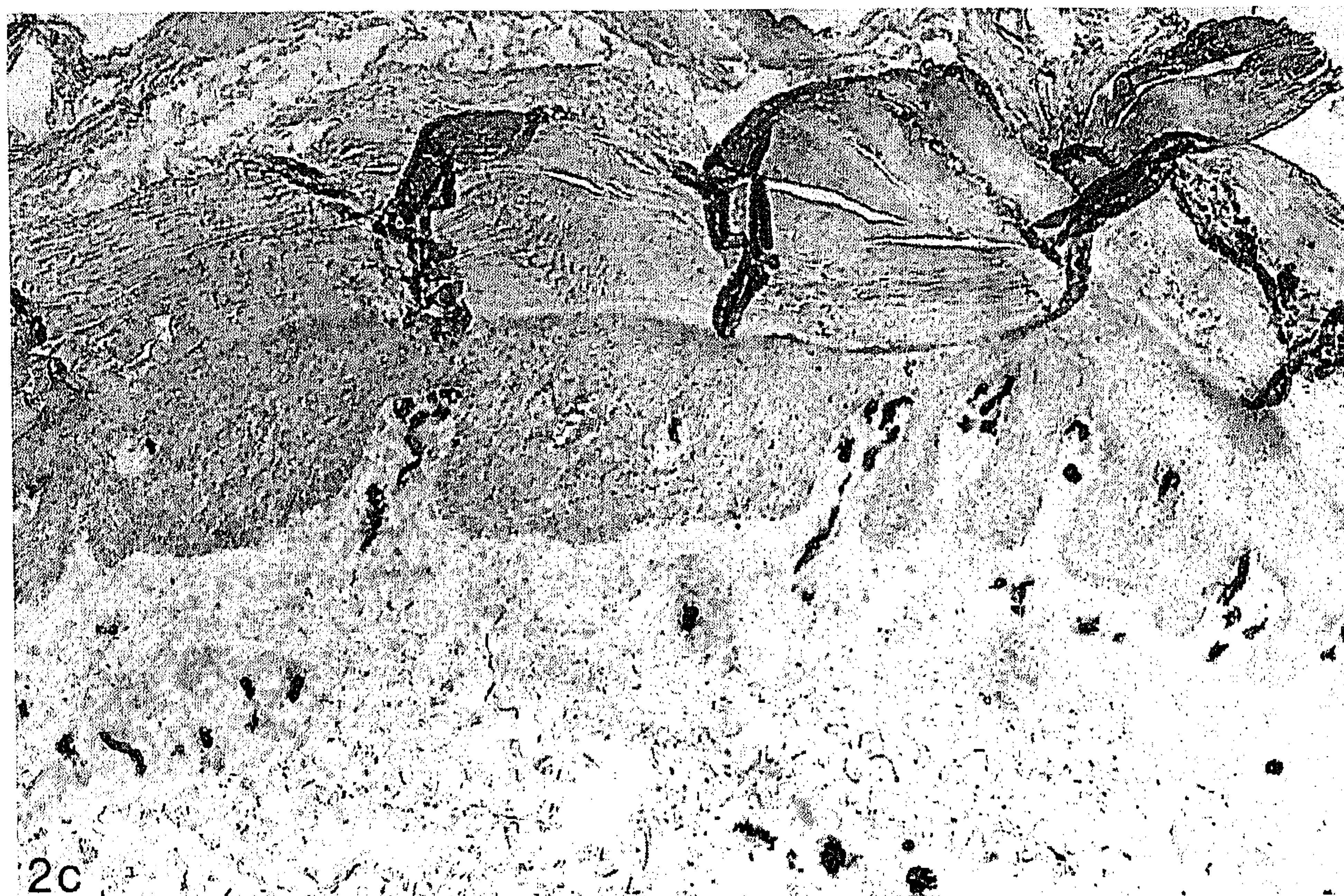


Fig. 2. Scoring (papillar level) of endoglin expression (125 \times): a. 1 = minimal staining, b. 2 = moderate staining, c. 3 = marked staining, d. 4 = extensive staining.



now found that at the papillar level of psoriatic uninvolved skin (Table 2) there was less endoglin expressing endothelium compared with normal skin ($P < 0.01$), suggesting less activated endothelium.

4. Discussion

In the normal skin the distribution of PAL-E and PN-E2 was for the greater part comparable. PAL-E and PN-E2 both showed staining that was

Table 2

Ratios of endoglin expression and PAL-E antigen expression in normal and psoriatic uninvolved skin

	Papillar	Subpapillar	Low dermal
Normal skin	0.9 ± 0.2	0.7 ± 0.1	0.9 ± 0.1
Psoriatic uninvolved skin	0.3 ± 0.2	0.6 ± 0.1	0.9 ± 0.2

restricted to endothelial cells except for some staining of the basal epidermis by PAL-E, as already described in [16]. In the clinically uninvolved skin both antibodies showed a decreased binding compared with normal skin, PN-E2 demonstrating the most pronounced decrease (Table 1).

In order to determine the levels of endothelial endoglin expression, the ratios of endoglin and PAL-E antigen expression were calculated. In the papillar region a relative decrease of endoglin expression was observed (see Table 2). Taking into account, however, the fact that PN-E2 stains the endothelial lining of both venous and arterial capillaries, venules, veins and probably also of lymphatic vessels, it seems not completely correct to relate it to PAL-E, which reacts predominantly with endothelial cells of medium and small sized veins and venules, but not at all or only weakly with those of arteries and arterioles and not with lymphatic endothelium.

As mentioned above, PN-E2 is a recently developed marker for endoglin, a RGD-containing glycoprotein predominantly expressed on the luminal membrane of endothelial cells, activated monocytes and macrophages and syncytiotrophoblasts. It has been shown that this antigen is considerably upregulated under inflammatory conditions such as psoriasis and in conditions with endothelial cell proliferation such as granulation tissue and cutaneous melanoma [12].

Endoglin seems to play a role in adhesion processes [13] and shares regions of sequence identity with betaglycan, a major binding protein for TGF- β . It binds TGF- β 1 and TGF- β 3 with high affinity, but fails to bind TGF- β 2 [14]. It is attractive to hypothesize that the large amount of endoglin in psoriatic involved skin acts as a scavenger by binding TGF- β and thus could modulate TGF- β inter-

action with its signaling receptors [15]. However, so far it cannot be excluded that endoglin might enhance TGF- β presentation under some conditions [14]. It is remotely possible that some peptides bind TGF- β and might modulate TGF- β activity, whereas other peptides leave TGF- β activity unaffected.

Wong et al. [19] found that after a single application of LTB₄ significantly fewer intraepidermal neutrophils were present in psoriatic uninvolved skin than in normal skin. Furthermore, repeated applications of LTB₄ gave significantly lower amounts of intraepidermal neutrophils than a single application [19]. These findings were confirmed by Lammers and Van De Kerkhof [20] and suggest some sort of restraining mechanism for the extravasation of polymorphonuclear leukocytes (PMN) in psoriatic uninvolved skin in vivo. TGF- β has a restraining influence on the extravasation of peripheral white bloodcells [15]. An increased availability of functional TGF- β in psoriatic uninvolved skin may contribute to this decreased extravasation of PMN's. In psoriatic uninvolved skin, the low amount of endoglin will be saturated with TGF- β sooner than the endoglin in normal skin. This could indeed mean that the remaining TGF- β can prevent further extravasation of white blood cells, which could explain the difference in responsiveness of normal and psoriatic skin to LTB₄ application. On the other hand, the increased expression of endoglin in lesional skin might prevent TGF- β availability and hence be a permissive factor for psoriatic inflammation.

Acknowledgements

We wish to acknowledge Ir. J.B. Boezeman for the statistical analyses and Dr. P.E.J. van Erp for the helpful discussions.

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